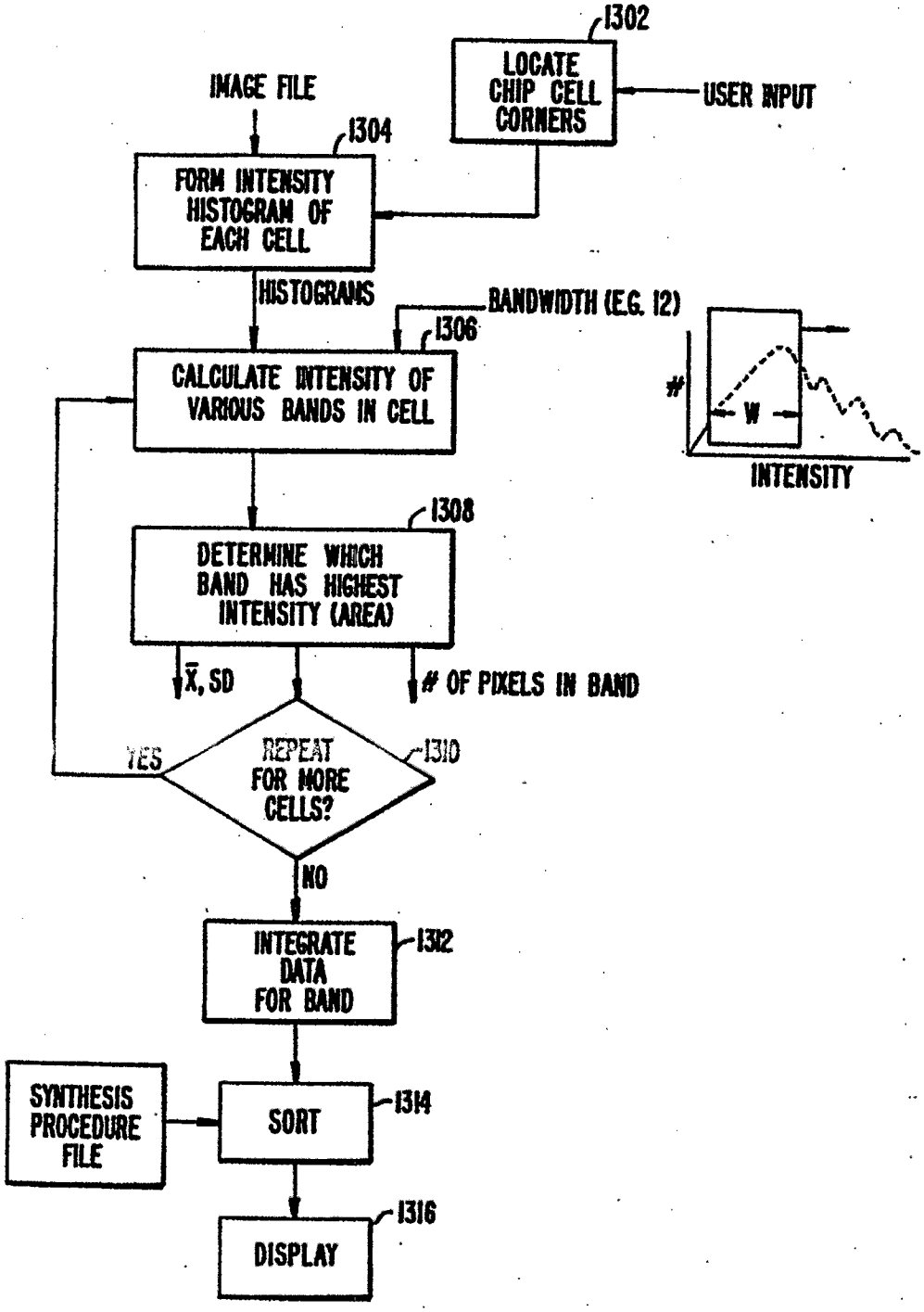
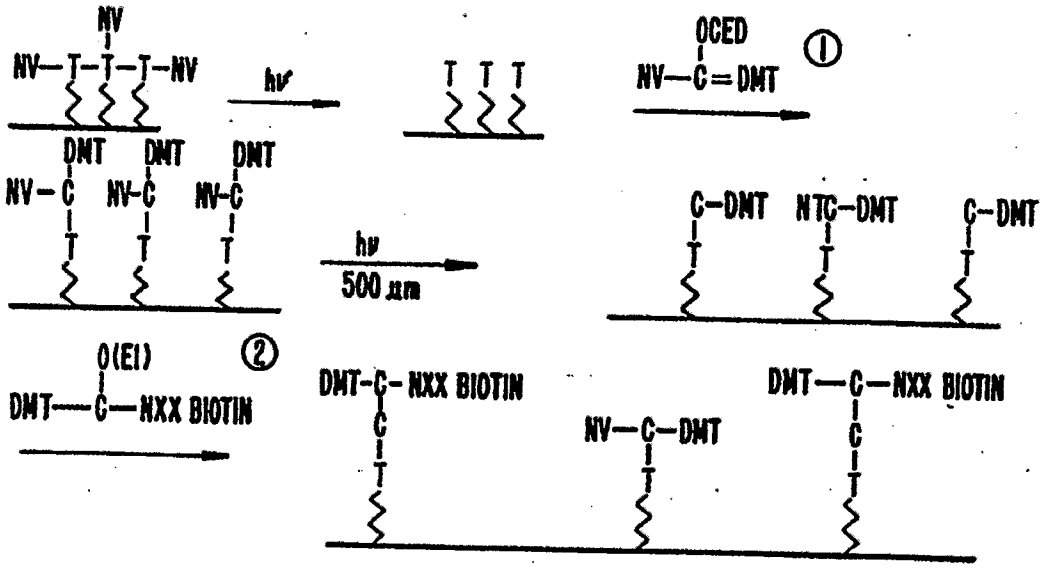

1302
LOCATE CHIP CELL CORNERS
USER INPUT
IMAGE FILE
1304
FORM INTENSITY HISTOGRAM OF EACH CELL
HISTOGRAMS
BANDWIDTH (E.G. 12)
1306
CALCULATE INTENSITY OF VARIOUS BANDS IN CELL
#
W
INTENSITY
1308
DETERMINE WHICH BAND HAS HIGHEST INTENSITY (AREA)
X̄, SD
OF PIXELS IN BAND
YES
REPEAT FOR MORE CELLS?
1310
NO
INTEGRATE DATA FOR BAND
1312
SYNTHESIS PROCEDURE FILE
SORT
1314
DISPLAY
1316

FIG. 12.


NV
NV—T—T—T—NV
hν
T T T
OCED
NV—C=DMT
①
DMT DMT DMT
NV—C NV-C NV-C
T T T
hν
500 nm
C-DMT NTC-DMT C-DMT
T T T
O(EI)
DMT—C—NXX BIOTIN
②
DMT-C-NXX BIOTIN
NV—C—DMT
DMT—C—NXX BIOTIN

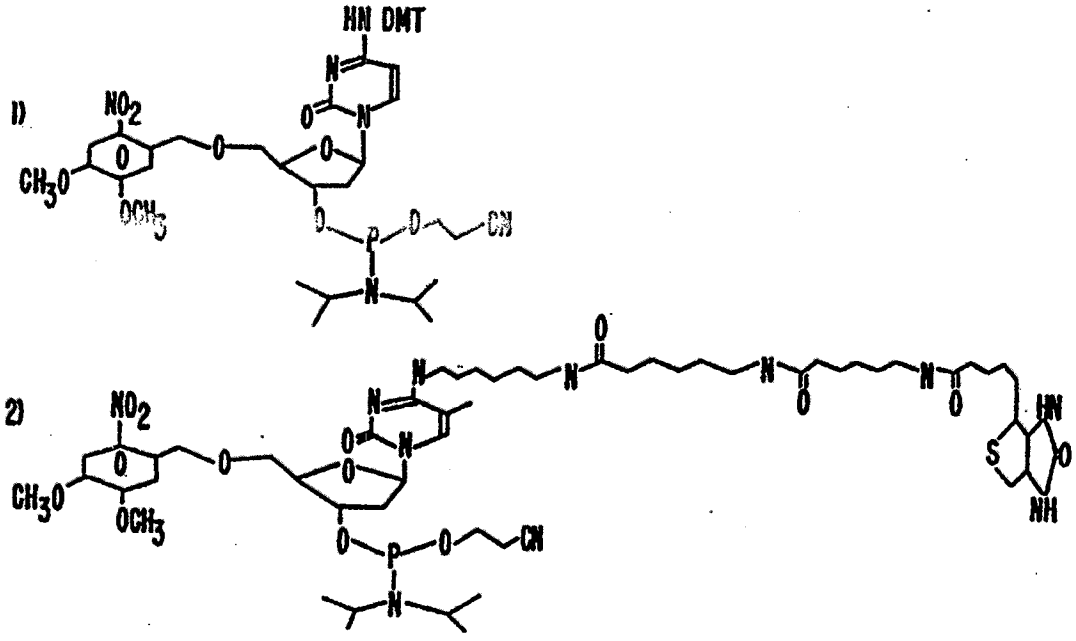

1)
HN DMT
NO2
CH3O
OCH3
CN
2)
NO2
CH3O
OCH3
CN
HN
NH

FIG. 13.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/08693

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): Please See Attached Sheet.
US CL : Please See Attached Sheet.

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	435/7.92, 7.94, 7.95, 961, 968, 973, 307; 536/26; 562/441; 436/518, 527, 807; 525/54.1, 54.11; 422/116, 131; 530/333, 334, 335, 336, 337; 935/88

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

AUTOMATED PATENT SYSTEM (APS); CHEMICAL ABSTRACTS

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
X,P/Y	WO, A, 90/15070 (Pirrung et al) 13 December 1990. See abstract; page 4, line 16-page 6, line 35; page 16, line 22-page 17, line 19; page 23, lines 1-28; page 24, lines 9-26; page 26, Table I; page 35, lines 19-35; page 41, lines 5-31; claims 1-46.	1-23,26,28-37,42-45/24-25,27,38-41,46-56
X/Y	D. McGillis, "Lithography", in VLSI TECHNOLOGY, published 1983 by McGraw-Hill Book Company (New York), pages 267-301. See pages 267-274.	3-6,8,9,11,12-16/1-2,7,10,17-27
X,P/Y	SCIENCE, vol. 251, issued 15 February 1991, S. Fodor et al., "Light-Directed, Spatially Addressable Parallel Chemical Synthesis", pages 767-773. See entire document.	3-10,12,14-22,26,28-37,42-47/1-2,11,13,23-25,27,38-41,46-56
X,P/Y	CHEMICAL ABSTRACTS, vol. 114, no. 15, issued 15 April 1991 (Columbus, Ohio, USA), S. Robertson et al., "A general and efficient route for chemical aminoacylation of transfer RNAs", see page 839, cols. 1-2, the abstract no. 143954x, J. Am. Chem. Soc., 1991, 113(7), 2722-9 (Eng). See entire abstract.	28-29,31-37/30

* Special categories of cited documents:[16]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
14 MARCH 1991	31 MAR 1992
International Searching Authority[1]	Signature of Authorized Officer[20]
ISA/US	Carol A. Spiegel

Form PCT/ISA/210 (second sheet)(May 1986) 8

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
X	CHEMICAL ABSTRACTS, vol. 112, no. 11, issued 12 March 1990 (Columbus, Ohio, USA), C. Shin et al., "Dehydrooligopeptides. XI. Facile synthesis of various kinds of dehydrodi- and tripeptides, and dehydroenkephalins containing Tyr residue by using N-carboxydehydrotyrosine anhydride", see page 818, col. 2, the abstract no. 99207p, Bull. Chem. Soc. Jpn. 1989, 62(4), 1127-35 (Eng)> See entire abstract.	50-56
XP/Y	WO, A, 91/07087 (Barrett et al), 30 May 1991. See abstract; page 3, line 20-page 4, line 14; page 6, lines 16-25; page 10, line 1-page 12, line 11; page 13, lines 32-33; page 24, lines 11-23; page 26, lines 1-16; Table 3; page 31, lines 20-28; page 35, lines 3-30.	46-49/1-37,42-45,50-56
Y	US, A, 4,517,338 (Urdea et al) 14 May 1985. See abstract; col. 3, lines 1-17 and col. 14, line 52-col. 15, line 3.	1-3
Y	PROCEEDINGS OF THE INDIAN NATIONAL SCIENCE ACADEMY, vol. 53, no. 6, issued 1987, V.K. Haridasan et al, "Peptide Synthesis Using Photolytically Cleavalable 2-Nitro-benzyloxycarbonyl protecting group", pages 717-728. See abstract; introduction, cpd (4); and page 727, lines 17-34.	3-21,28-37,42-49
Y	WO, A, 84/03564 (Geysen et al) 13 September 1984. See page 12, line 12-page 3, line 3; page 5, line 23-page 8, line 25; page 11, line 26-page 12, line 20.	3-27
Y	US, A, 4,562,157 (Lowe et al) 31 December 1985. See col. 3, line 3-col. 4, line 15; and col. 9, lines 18-22.	3-27
Y	WO, A, 90/04652 (Macevicz) 03 May 1990. See page 5, line 17-page 6, line 9; page 9, lines 19-35; page 15, line 19-page 20, line 28; Figure 1.	3-4,9-13,15-7,22,26-27
Y	US, A, 4,762,881 (Kauer) 09 August 1988. See col. 1, line 1-col. 3, line 52.	3-22
A	EP, A, 0,228,310 (Sherrington et al) 26 October 1988. See entire document.	1-27
A	US, A, 3,849,137 (Barzynski et al) 19 November 1974. See entire document.	1-56
A	US, A, 4,631,211 (Houghten) 23 December 1986. See entire document.	1-27
A	APPLIED PHYSICS LETTERS, vol. 31, no. 7, issued 01 October 1977, D. Flanders et al., "A new interferometric alignment technique", pages 426-429. See entire document.	1-25

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X,P/Y	CHEMICAL ABSTRACTS, vol. 114, no. 23, issued 10 June 1991 (Columbus, Ohio USA), M. Iwamura et al, "1-x-Diazobenzyl) pyrene: a reagent for photloabile and fluorescent protection of carboxyl groups of amino acids and peptides", see page 827, col. 1, the abstract no. 229349r, Synlett 1991, (1), 35-6 (Eng). See entire abstract.	38-40/41
X	JOURNAL OF THE AMERICAN SOCIETY, vol. 92, no. 21, issued 21 October 1970, A. Patchornik et al., "Photosensitive Protecting Groups", pages 6333-6335. See page 6334, Scheme I.	42-45

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _, because they relate to subject matter (1) not required to be searched by this Authority, namely:

2. ☐ Claim numbers _, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:

3. ☐ Claim numbers _, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PREVIOUS SHEETS

I. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A01N 1/02; C12Q 1/00; G01N 33/566, 33/543; B01J 19/00; C07D 471/02, 235/00, 473/00, 235/30; C07K 1/04, 17/06, 17/14

I. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.92, 7.94, 7.95, 961, 968, 973, 307; 536/26; 562/441; 436/518, 527, 807; 525/54.1, 54.11; 422/116, 131; 530/333, 334, 335, 336, 337; 935/88

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
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(11) International Publication Number: **WO 90/15070**

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(71) Applicant: AFFYMAX TECHNOLOGIES N.V. [NL/NL]; Van Boshuizenstraat 12, NL-1083 BA Amsterdam (NL).

(72) Inventors: PIRRUNG, Michael, C. ; 3421 Cottonwood, Durham, NC 27707 (US). READ, J., Leighton ; 1001 Ramona, Palo Alto, CA 94301 (US). FODOR, Stephen, P., A. ; 817 Wintergreen Way, Palo Alto, CA 94303 (US). STRYER, Lubert ; 843 Sonoma Terrace, Stanford, CA 94305 (US).

(74) Agent: SMULDERS, TH., A., H., J.; Vereenigde Octrooibureaux, Nieuwe Parklaan 107, NL-2587 BP The Hague (NL).

(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE*, DE (European patent)*, DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent).

Published
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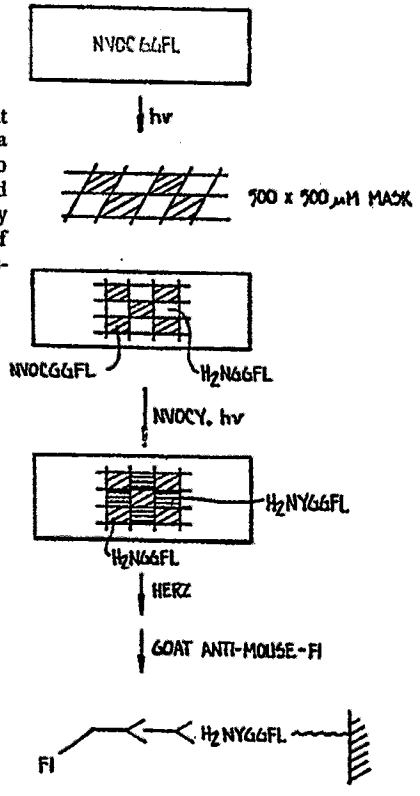
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: VERY LARGE SCALE IMMOBILIZED PEPTIDE SYNTHESIS

(57) Abstract

A method and device for preparing desired sequences on a substrate at known locations. Known locations (10) of a substrate (2) are irradiated by way of a mask (8) so as to activate a material (4) for binding. The substrate is then exposed to a first material (12) for binding thereto. Second locations (14) are then irradiated through a mask and exposed to a second material (16). A variety of sequences may be formed through selective irradiation of the substrate followed by application of selected materials. A reactor system and fluorescence detection system are also disclosed.



* See back of page

DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

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VERY LARGE SCALE IMMOBILIZED PEPTIDE SYNTHESIS

BACKGROUND OF THE INVENTION

The present inventions relate to the synthesis and placement materials at known locations. In particular, one embodiment of the inventions provides a method and associated apparatus for preparing diverse chemical sequences at known locations on a single substrate surface. The inventions may be applied, for example, in the field of preparation of oligomer, peptide, nucleic acid, oligosaccharide, phospholipid, polymer, or drug congener preparation, especially to create sources of chemical diversity for use in screening for biological activity.

The relationship between structure and activity of molecules is a fundamental issue in the study of biological systems. Structure-activity relationships are important in understanding, for example, the function of enzymes, the ways in which cells communicate with each other, as well as cellular control and feedback systems.

Certain macromolecules are known to interact and bind to other molecules having a very specific three-dimensional spatial and electronic distribution. Any large molecule having such specificity can be considered a receptor, whether it is an enzyme catalyzing hydrolysis of a metabolic intermediate, a cell-surface protein mediating membrane transport of ions, a glycoprotein serving to identify a particular cell to its neighbors,

an IgG-class antibody circulating in the plasma, an oligonucleotide sequence of DNA in the nucleus, or the like. The various molecules which receptors selectively bind are known as ligands.

Many assays are available for measuring the binding affinity of known receptors and ligands, but the information which can be gained from such experiments is often limited by the number and type of ligands which are available. Novel ligands are sometimes discovered by chance or by application of new techniques for the elucidation of molecular structure, including x-ray crystallographic analysis and recombinant genetic techniques for proteins.

Small peptides are an exemplary system for exploring the relationship between structure and function in biology. A peptide is a sequence of amino acids. When the twenty naturally occurring amino acids are condensed into polymeric molecules they form a wide variety of three-dimensional configurations, each resulting from a particular amino acid sequence and solvent condition. The number of possible pentapeptides of the 20 naturally occurring amino acids, for example, is 20^5 or 3.2 million different peptides. The likelihood that molecules of this size might be useful in receptor-binding studies is supported by epitope analysis studies showing that some antibodies recognize sequences as short as a few amino acids with high specificity. Furthermore, the average molecular weight of amino acids puts small peptides in the size range of many currently useful pharmaceutical products.

Pharmaceutical drug discovery is one type of research which relies on such a study of structure-activity relationships. In most cases, contemporary pharmaceutical research can be described as the process of discovering novel ligands with desirable patterns of specificity for biologically important receptors.

Another example is research to discover new compounds for use in agriculture, such as pesticides and herbicides.

Sometimes, the solution to a rational process of designing ligands is difficult or unyielding. Prior methods of preparing large numbers of different polymers have been painstakingly slow when used at a scale sufficient to permit effective rational or random screening. For example, the "Merrifield" method (J. Am. Chem. Soc. (1963) 85:2149-2154, which is incorporated herein by reference for all purposes) has been used to synthesize peptides on a solid support. In the Merrifield method, an amino acid is covalently bonded to a support made of an insoluble polymer. Another amino acid with an alpha protected group is reacted with the covalently bonded amino acid to form a dipeptide. After washing, the protective group is removed and a third amino acid with an alpha protective group is added to the dipeptide. This process is continued until a peptide of a desired length and sequence is obtained. Using the Merrifield method, it is not economically practical to synthesize more than a handful of peptide sequences in a day.

To synthesize larger numbers of polymer sequences, it has also been proposed to use a series of reaction vessels for polymer synthesis. For example, a tubular reactor system may be used to synthesize a linear polymer on a solid phase support by automated sequential addition of reagents. This method still does not enable the synthesis of a sufficiently large number of polymer sequences for effective economical screening.

Methods of preparing a plurality of polymer sequences are also known in which a foraminous container encloses a known quantity of reactive particles, the particles being larger in size than foramina of the container. The containers may be selectively reacted with desired materials to synthesize desired sequences of product molecules. As with other methods known in the

art, this method cannot practically be used to synthesize a sufficient variety of polypeptides for effective screening.

Other techniques have also been described. These methods include the synthesis of peptides on 96 plastic pins which fit the format of standard microtiter plates. Unfortunately, while these techniques have been somewhat useful, substantial problems remain. For example, these methods continue to be limited in the diversity of sequences which can be economically synthesized and screened.

From the above, it is seen that an improved method and apparatus for synthesizing a variety of chemical sequences at known locations is desired.

SUMMARY OF THE INVENTION

An improved method and apparatus for the preparation of a variety of polymers is disclosed.

In one preferred embodiment, linker molecules are provided on a substrate. A terminal end of the linker molecules is provided with a reactive functional group protected with a photoremovable protective group. Using lithographic methods, the photoremovable protective group is exposed to light and removed from the linker molecules in first selected regions. The substrate is then washed or otherwise contacted with a first monomer that reacts with exposed functional groups on the linker molecules. In a preferred embodiment, the monomer is an amino acid containing a photoremovable protective group at its amino or carboxy terminus and the linker molecule terminates in an amino or carboxy acid group bearing a photoremovable protective group.

A second set of selected regions is, thereafter, exposed to light and the photoremovable protective group on the linker molecule/protected amino acid is removed at the second set of regions. The substrate is then contacted with a second monomer

containing a photoremovable protective group for reaction with exposed functional groups. This process is repeated to selectively apply monomers until polymers of a desired length and desired chemical sequence are obtained. Photolabile groups are then optionally removed and the sequence is, thereafter, optionally capped. Side chain protective groups, if present, are also removed.

By using the lithographic techniques disclosed herein, it is possible to direct light to relatively small and precisely known locations on the substrate. It is, therefore, possible to synthesize polymers of a known chemical sequence at known locations on the substrate.

The resulting substrate will have a variety of uses including, for example, screening large numbers of polymers for biological activity. To screen for biological activity, the substrate is exposed to one or more receptors such as antibody whole cells, receptors on vesicles, lipids, or any one of a variety of other receptors. The receptors are preferably labeled with, for example, a fluorescent marker, radioactive marker, or a labeled antibody reactive with the receptor. The location of the marker on the substrate is detected with, for example, photon detection or autoradiographic techniques. Through knowledge of the sequence of the material at the location where binding is detected, it is possible to quickly determine which sequence binds with the receptor and, therefore, the technique can be used to screen large numbers of peptides. Other possible applications of the inventions herein include diagnostics in which various antibodies for particular receptors would be placed on a substrate and, for example, blood sera would be screened for immune deficiencies. Still further applications include, for example, selective "doping" of organic materials in semiconductor devices, and the like.

In connection with one aspect of the invention an improved reactor system for synthesizing polymers is also disclosed. The reactor system includes a substrate mount which engages a substrate around a periphery thereof. The substrate mount provides for a reactor space between the substrate and the mount through or into which reaction fluids are pumped or flowed. A mask is placed on or focused on the substrate and illuminated so as to deprotect selected regions of the substrate in the reactor space. A monomer is pumped through the reactor space or otherwise contacted with the substrate and reacts with the deprotected regions. By selectively deprotecting regions on the substrate and flowing predetermined monomers through the reactor space, desired polymers at known locations may be synthesized.

Improved detection apparatus and methods are also disclosed. The detection method and apparatus utilize a substrate having a large variety of polymer sequences at known locations on a surface thereof. The substrate is exposed to a fluorescently labeled receptor which binds to one or more of the polymer sequences. The substrate is placed in a microscope detection apparatus for identification of locations where binding takes place. The microscope detection apparatus includes a monochromatic or polychromatic light source for directing light at the substrate, means for detecting fluoresced light from the substrate, and means for determining a location of the fluoresced light. The means for detecting light fluoresced on the substrate may in some embodiments include a photon counter. The means for determining a location of the fluoresced light may include an x/y translation table for the substrate. Translation of the slide and data collection are recorded and managed by an appropriately programmed digital computer.

A further understanding of the nature and advantages of the inventions herein may be realized by reference to the remaining portions of the specification and the attached drawings.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 illustrates masking and irradiation of a substrate at a first location. The substrate is shown in cross-section;

Fig. 2 illustrates the substrate after application of a monomer "A";

Fig. 3 illustrates irradiation of the substrate at a second location;

Fig. 4 illustrates the substrate after application of monomer "B";

Fig. 5 illustrates irradiation of the "A" monomer;

Fig. 6 illustrates the substrate after a second application of "B";

Fig. 7 illustrates a completed substrate;

Figs. 8A and 8B illustrate alternative embodiments of a reactor system for forming a plurality of polymers on a substrate;

Fig. 9 illustrates a detection apparatus for locating fluorescent markers on the substrate;

Figs. 10A-10M illustrate the method as it is applied to the production of the trimers of monomers "A" and "B";

Figs. 11A, 11B, and 11C are fluorescence traces for standard fluorescent beads;

Figs. 12A and 12B are fluorescence curves for NVOC slides not exposed and exposed to light respectively;

Figs. 13A and 13B illustrate formation of a slide with a checkerboard pattern of YGGFL and GGFL exposed to labeled Herz antibody; and

Figs. 14A and 14B illustrate the mapping of sixteen sequences synthesized on two different glass slides.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

CONTENTS

CONTENTS
(Cont'd)

I. Glossary

The following terms are intended to have the following general meanings as they are used herein:

1. Complementary: Refers to the topological compatibility or matching together of interacting surfaces of a ligand molecule and its receptor. Thus, the receptor and its ligand can be described as complementary, and furthermore, the contact surface characteristics are complementary to each other.

2. Epitope: The portion of an antigen molecule which is delineated by the area of interaction with the subclass of receptors known as antibodies.

3. Ligand: A ligand is a molecule that is recognized by a particular receptor. Examples of ligands that can be investigated by this invention include, but are not restricted to, agonists and antagonists for cell membrane receptors, toxins and venoms, viral epitopes, hormones (e.g., opiates, steroids, etc.), hormone receptors, peptides, enzymes, enzyme substrates, cofactors, drugs, lectins, sugars,

oligonucleotides, nucleic acids, oligosaccharides, proteins, and monoclonal antibodies.

4. Monomer: A member of the set of small molecules which can be joined together to form a polymer. The set of monomers includes but is not restricted to, for example, the set of common L-amino acids, the set of D-amino acids, the set of synthetic amino acids, the set of nucleotides and the set of pentoses and hexoses. As used herein, monomers refers to any member of a basis set for synthesis of a polymer. For example, dimers of L-amino acids form a basis set of 400 monomers for synthesis of polypeptides. Different basis sets of monomers may be used at successive steps in the synthesis of a polymer.

5. Peptide: A polymer in which the monomers are alpha amino acids and which are joined together through amide bonds and alternatively referred to as a polypeptide. In the context of this specification it should be appreciated that the amino acids may be the L-optical isomer or the D-optical isomer. Peptides are more than two amino acid monomers long, and often more than 20 amino acid monomers long. Standard abbreviations for amino acids are used (e.g., P for proline). These abbreviations are included in Stryer, Biochemstry, Third Ed., 1988, which is incorporated herein by reference for all purposes.

6. Radiation: Energy which may be selectively applied including energy having a wavelength of between 10^{-14} and 10^{4} meters including, for example, electron beam radiation, gamma radiation, x-ray radiation, ultra-violet radiation, visible light, infrared radiation,

microwave radiation, and radio waves. "Irradiation" refers to the application of radiation to a surface.

7. Receptor: A molecule that has an affinity for a given ligand. Receptors may be naturally-occuring or manmade molecules. Also, they can be employed in their unaltered state or as aggregates with other species. Receptors may be attached, covalently or noncovalently, to a binding member, either directly or via a specific binding substance. Examples of receptors which can be employed by this invention include, but are not restricted to, antibodies, cell membrane receptors, monoclonal antibodies and antisera reactive with specific antigenic determinants (such as on viruses, cells or other materials), drugs, polynucleotides, nucleic acids, peptides, cofactors, lectins, sugars, polysaccharides, cells, cellular membranes, and organelles. Receptors are sometimes referred to in the art as anti-ligands. As the term receptors is used herein, no difference in meaning is intended. A "Ligand Receptor Pair" is formed when two macromolecules have combined through molecular recognition to form a complex.

Other examples of receptors which can be investigated by this invention include but are not restricted to:

a) Microorganism receptors: Determination of ligands which bind to receptors, such as specific transport proteins or enzymes essential to survival of microorganisms, is useful in a new class of antibiotics. Of particular value would be antibiotics against opportunistic fungi, protozoa, and those bacteria resistant to the antibiotics in current use.

b) Enzymes: For instance, the binding site of enzymes such as the enzymes responsible for cleaving neurotransmitters; determination of ligands which bind to certain receptors to modulate the action of the enzymes which cleave the different neurotransmitters is useful in the development of drugs which can be used in the treatment of disorders of neurotransmission.

c) Antibodies: For instance, the invention may be useful in investigating the ligand-binding site on the antibody molecule which combines with the epitope of an antigen of interest; determining a sequence that mimics an antigenic epitope may lead to the development of vaccines of which the immunogen is based on one or more of such sequences or lead to the development of related diagnostic agents or compounds useful in therapeutic treatments such as for auto-immune diseases (e.g., by blocking the binding of the "self" antibodies).

d) Nucleic Acids: Sequences of nucleic acids may be synthesized to establish DNA or RNA binding sequences.

e) Catalytic Polypeptides: Polymers, preferably polypeptides, which are capable of promoting a chemical reaction involving the conversion of one or more reactants to one or more products. Such polypeptides generally include a binding site specific for at least one reactant or reaction intermediate and an active functionality proximate to the binding site, which functionality is capable of chemically modifying the bound reactant. Catalytic polypeptides are described in, for example, U.S. application Serial No. 404,920, which

is incorporated herein by reference for all purposes.

f) Hormone receptors: For instance, the receptors for insulin and growth hormone. Determination of the ligands which bind with high affinity to a receptor is useful in the development of, for example, an oral replacement of the daily injections which diabetics must take to relieve the symptoms of diabetes, and in the other case, a replacement for the scarce human growth hormone which can only be obtained from cadavers or by recombinant DNA technology. Other examples are the vasoconstrictive hormone receptors; determination of those ligands which bind to a receptor may lead to the development of drugs to control blood pressure.

g) Opiate receptors: Determination of ligands which bind to the opiate receptors in the brain is useful in the development of less-addictive replacements for morphine and related drugs.

8. Substrate: A material having a rigid or semi-rigid surface. In many embodiments, at least one surface of the substrate will be substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different polymers with, for example, wells, raised regions, etched trenches, or the like. According to other embodiments, small beads may be provided on the surface which may be released upon completion of the synthesis.

9. Protective Group: A material which is bound to a monomer unit and which may be spatially removed upon selective exposure to an activator such as electromagnetic radiation. Examples of protective groups with utility herein include Nitroveratryloxy

carbonyl, Nitrobenzyloxy carbonyl, Dimethyl dimethoxybenzyloxy carbonyl, 5-Bromo-7-nitroindolinyl, o-Hydroxy-α-methyl cinnamoyl, and 2-Oxymethylene anthraquinone. Other examples of activators include ion beams, electric fields, magnetic fields, electron beams, x-ray, and the like.

10. Predefined Region: A predefined region is a localized area on a surface which is, was, or is intended to be activated for formation of a polymer. The predefined region may have any convenient shape, e.g., circular, rectangular, elliptical, wedge-shaped, etc. For the sake of brevity herein, "predefined regions" are sometimes referred to simply as "regions."

11. Substantially Pure: A polymer is considered to be "substantially pure" within a predefined region of a substrate when it exhibits characteristics that distinguish it from other predefined regions. Typically, purity will be measured in terms of biological activity or function as a result of uniform sequence. Such characteristics will typically be measured by way of binding with a selected ligand or receptor.

II. General

The present invention provides methods and apparatus for the preparation and use of a substrate having a plurality of polymer sequences in predefined regions. The invention is described herein primarily with regard to the preparation of molecules containing sequences of amino acids, but could readily be applied in the preparation of other polymers. Such polymers include, for example, both linear and cyclic polymers of nucleic acids, polysaccharides, phospholipids, and

peptides having either α-, β-, or ω-amino acids, heteropolymers in which a known drug is covalently bound to any of the above, polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, polyacetates, or other polymers which will be apparent upon review of this disclosure. In a preferred embodiment, the invention herein is used in the synthesis of peptides.

The prepared substrate may, for example, be used in screening a variety of polymers as ligands for binding with a receptor, although it will be apparent that the invention could be used for the synthesis of a receptor for binding with a ligand. The substrate disclosed herein will have a wide variety of other uses. Merely by way of example, the invention herein can be used in determining peptide and nucleic acid sequences which bind to proteins, finding sequence-specific binding drugs, identifying epitopes recognized by antibodies, and evaluation of a variety of drugs for clinical and diagnostic applications, as well as combinations of the above.

The invention preferably provides for the use of a substrate "S" with a surface. Linker molecules "L" are optionally provided on a surface of the substrate. The purpose of the linker molecules, in some embodiments, is to facilitate receptor recognition of the synthesized polymers.

Optionally, the linker molecules may be chemically protected for storage purposes. A chemical storage protective group such as t-BOC (t-butoxycarbonyl) may be used in some embodiments. Such chemical protective groups would be chemically removed upon exposure to, for example, acidic solution and would serve to protect the surface during storage and be removed prior to polymer preparation.

On the substrate or a distal end of the linker molecules, a functional group with a protective group P_0

is provided. The protective group P_0 may be removed upon exposure to radiation, electric fields, electric currents, or other activators to expose the functional group.

In a preferred embodiment, the radiation is ultraviolet (UV), infrared (IR), or visible light. As more fully described below, the protective group may alternatively be an electrochemically-sensitive group which may be removed in the presence of an electric field. In still further alternative embodiments, ion beams, electron beams, or the like may be used for deprotection.

In some embodiments, the exposed regions and, therefore, the area upon which each distinct polymer sequence is synthesized are smaller than about 1 cm^2 or less than 1 mm^2. In preferred embodiments the exposed area is less than about 10,000 μm^2 or, more preferably, less than 100 μm^2 and may, in some embodiments, encompass the binding site for as few as a single molecule. Within these regions, each polymer is preferably synthesized in a substantially pure form.

Concurrently or after exposure of a known region of the substrate to light, the surface is contacted with a first monomer unit M_1 which reacts with the functional group which has been exposed by the deprotection step. The first monomer includes a protective group P_1. P_1 may or may not be the same as P_0.

Accordingly, after a first cycle, known first regions of the surface may comprise the sequence:

$$S\text{-}L\text{-}M_1\text{-}P_1$$

while remaining regions of the surface comprise the sequence:

$$S\text{-}L\text{-}P_0.$$

Thereafter, second regions of the surface (which may include the first region) are exposed to light and contacted with a second monomer M_2 (which may or may not be the same as M_1) having a protective group P_2. P_2 may or may not be the same as P_0 and P_1. After this second cycle, different regions of the substrate may comprise one or more of the following sequences:

$$S\text{-}L\text{-}M_1\text{-}M_2\text{-}P_2$$
$$S\text{-}L\text{-}M_2\text{-}P_2$$
$$S\text{-}L\text{-}M_1\text{-}P_1 \text{ and/or}$$
$$S\text{-}L\text{-}P_0.$$

The above process is repeated until the substrate includes desired polymers of desired lengths. By controlling the locations of the substrate exposed to light and the reagents exposed to the substrate following exposure, the location of each sequence will be known.

Thereafter, the protective groups are removed from some or all of the substrate and the sequences are, optionally, capped with a capping unit C. The process results in a substrate having a surface with a plurality of polymers of the following general formula:

$$S\text{-}[L]\text{-}(M_i)\text{-}(M_j)\text{-}(M_k)\ldots(M_x)\text{-}[C]$$

where square brackets indicate optional groups, and $M_i \ldots M_x$ indicates any sequence of monomers. The number of monomers could cover a wide variety of values, but in a preferred embodiment they will range from 2 to 100.

In some embodiments a plurality of locations on the substrate polymers are to contain a common monomer subsequence. For example, it may be desired to synthesize a sequence $S\text{-}M_1\text{-}M_2\text{-}M_3$ at first locations and a sequence $S\text{-}M_4\text{-}M_2\text{-}M_3$ at second locations. The process would commence with irradiation of the first locations

followed by contacting with M_1-P, resulting in the sequence S-M_1-P at the first location. The second locations would then be irradiated and contacted with M_4-P, resulting in the sequence S-M_4-P at the second locations. Thereafter both the first and second locations would be irradiated and contacted with the dimer M_2-M_3, resulting in the sequence S-M_1-M_2-M_3 at the first locations and S-M_4-M_2-M_3 at the second locations. Of course, common subsequences of any length could be utilized including those in a range of 2 or more monomers, 2 to 100 monomers, 2 to 20 monomers, and a most preferred range of 2 to 3 monomers.

According to other embodiments, a set of masks is used for the first monomer layer and, thereafter, varied light wavelengths are used for selective deprotection. For example, in the process discussed above, first regions are first exposed through a mask and reacted with a first monomer having a first protective group P_1, which is removable upon exposure to a first wavelength of light (e.g., IR). Second regions are masked and reacted with a second monomer having a second protecive group P_2, which is removable upon exposure to a second wavelength of light (e.g., UV). Thereafter, masks become unnecessary in the synthesis because the entire substrate may be exposed alternatively to the first and second wavelengths of light in the deprotection cycle.

The polymers prepared on a substrate according to the above methods will have a variety of uses including, for example, screening for biological activity. In such screening activities, the substrate containing the sequences is exposed to an unlabeled or labeled receptor such as an antibody, receptor on a cell, phospholipid vesicle, or any one of a variety of other receptors. In one preferred embodiment the polymers are exposed to a first, unlabeled receptor of interest and, thereafter, exposed to a labeled receptor-specific recognition element, which is, for example, an antibody. This

process will provide signal amplification in the detection stage.

The receptor molecules may bind with one or more polymers on the substrate. The presence of the labeled receptor and, therefore, the presence of a sequence which binds with the receptor is detected in a preferred embodiment through the use of autoradiography, detection of fluorescence with a charge-coupled device, fluorescence microscopy, or the like. The sequence of the polymer at the locations where the receptor binding is detected may be used to determine all or part of a sequence which is complementary to the receptor.

Use of the invention herein is illustrated primarily with reference to screening for biological activity. The invention will, however, find many other uses. For example, the invention may be used in information storage (e.g., on optical disks), production of molecular electronic devices, production of stationary phases in separation sciences, production of dyes and brightening agents, photography, and in immobilization of cells, proteins, lectins, nucleic acids, polysaccharides and the like in patterns on a surface via molecular recognition of specific polymer sequences. By synthesizing the same compound in adjacent, progressively differing concentrations, a gradient will be established to control chemotaxis or to develop diagnostic dipsticks which, for example, titrate an antibody against an increasing amount of antigen. By synthesizing several catalyst molecules in close proximity, more efficient multistep conversions may be achieved by "coordinate immobilization." Coordinate immobilization also may be used for electron transfer systems, as well as to provide both structural integrity and other desirable properties to materials such as lubrication, wetting, etc.

According to alternative embodiments, molecular biodistribution or pharmacokinetic properties may be examined. For example, to assess resistance to

intestinal or serum proteases, polymers may be capped with a fluorescent tag and exposed to biological fluids of interest.

III. Polymer Synthesis

Fig. 1 illustrates one embodiment of the invention disclosed herein in which a substrate 2 is shown in cross-section. Essentially, any conceivable substrate may be employed in the invention. The substrate may be biological, nonbiological, organic, inorganic, or a combination of any of these, existing as particles, strands, precipitates, gels, sheets, tubing, spheres, containers, capillaries, pads, slices, films, plates, slides, etc. The substrate may have any convenient shape, such as a disc, square, sphere, circle, etc. The substrate is preferably flat but may take on a variety of alternative surface configurations. For example, the substrate may contain raised or depressed regions on which the synthesis takes place. The substrate and its surface preferably form a rigid support on which to carry out the reactions described herein. The substrate and its surface is also chosen to provide appropriate light-absorbing characteristics. For instance, the substrate may be a polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO_2, SiN_4, modified silicon, or any one of a wide variety of gels or polymers such as (poly)tetrafluoro-ethylene, (poly)vinylidenedifluoride, polystyrene, polycarbonate, or combinations thereof. Other substrate materials will be readily apparent to those of skill in the art upon review of this disclosure. In a preferred embodiment the substrate is flat glass or single-crystal silicon with surface relief features of less than 10 Å.

According to some embodiments, the surface of the substrate is etched using well known techniques to provide for desired surface features. For example, by way of the formation of trenches, v-grooves, mesa

structures, or the like, the synthesis regions may be more closely placed within the focus point of impinging light, be provided with reflective "mirror" structures for maximization of light collection from fluorescent sources, or the like.

Surfaces on the solid substrate will usually, though not always, be composed of the same material as the substrate. Thus, the surface may be composed of any of a wide variety of materials, for example, polymers, plastics, resins, polysaccharides, silica or silica-based materials, carbon, metals, inorganic glasses, membranes, or any of the above-listed substrate materials. In some embodiments the surface may provide for the use of caged binding members which are attached firmly to the surface of the substrate in accord with the teaching of copending application Serial No. 404,920, previously incorporated herein by reference. Preferably, the surface will contain reactive groups, which could be carboxyl, amino, hydroxyl, or the like. Most preferably, the surface will be optically transparent and will have surface Si-OH functionalities, such as are found on silica surfaces.

The surface 4 of the substrate is preferably provided with a layer of linker molecules 6, although it will be understood that the linker molecules are not required elements of the invention. The linker molecules are preferably of sufficient length to permit polymers in a completed substrate to interact freely with molecules exposed to the substrate. The linker molecules should be 6-50 atoms long to provide sufficient exposure. The linker molecules may be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof. Other linker molecules may be used in light of this disclsoure.

According to alternative embodiments, the linker molecules are selected based upon their hydrophilic/hydrophobic properties to improve

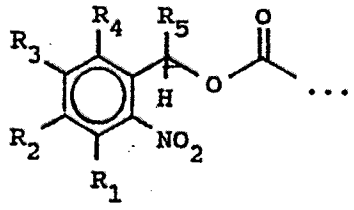
presentation of synthesized polymers to certain receptors. For example, in the case of a hydrophilic receptor, hydrophilic linker molecules will be preferred so as to permit the receptor to more closely approach the synthesized polymer.

According to another alternative embodiment, linker molecules are also provided with a photocleavable group at an intermediate position. The photocleavable group is preferably cleavable at a wavelength different from the protective group. This enables removal of the various polymers following completion of the synthesis by way of exposure to the different wavelengths of light.

The linker molecules can be attached to the substrate via carbon-carbon bonds using, for example, (poly)trifluorochloroethylene surfaces, or preferably, by siloxane bonds (using, for example, glass or silicon oxide surfaces). Siloxane bonds with the surface of the substrate may be formed in one embodiment via reactions of linker molecules bearing trichlorosilyl groups. The linker molecules may optionally be attached in an ordered array, i.e., as parts of the head groups in a polymerized Langmuir Blodgett film. In alternative embodiments, the linker molecules are adsorbed to the surface of the substrate.

The linker molecules and monomers used herein are provided with a functional group to which is bound a protective group. Preferably, the protective group is on the distal or terminal end of the linker molecule opposite the substrate. The protective group may be either a negative protective group (i.e., the protective group renders the linker molecules less reactive with a monomer upon exposure) or a positive protective group (i.e., the protective group renders the linker molecules more reactive with a monomer upon exposure). In the case of negative protective groups an additional step of reactivation will be required. In some embodiments, this will be done by heating.

The protective group on the linker molecules may be selected from a wide variety of positive light-reactive groups preferably including nitro aromatic compounds such as o-nitrobenzyl derivatives or benzylsulfonyl. In a preferred embodiment, 6-nitroveratryloxycarbonyl (NVOC), 2-nitrobenzyloxycarbonyl (NBOC) or α,α-dimethyl-dimethoxybenzyloxycarbonyl (DDZ) is used. In one embodiment, a nitro aromatic compound containing a benzylic hydrogen ortho to the nitro group is used, i.e., a chemical of the form:



where R_1 is alkoxy, alkyl, halo, aryl, alkenyl, or hydrogen; R_2 is alkoxy, alkyl, halo, aryl, nitro, or hydrogen; R_3 is alkoxy, alkyl, halo, nitro, aryl, or hydrogen; R_4 is alkoxy, alkyl, hydrogen, aryl, halo, or nitro; and R_5 is alkyl, alkynyl, cyano, alkoxy, hydrogen, halo, aryl, or alkenyl. Other materials which may be used include o-hydroxy-α-methyl cinnamoyl derivatives. Photoremovable protective groups are described in, for example, Patchornik, J. Am. Chem. Soc. (1970) 92:6333 and Amit et al., J. Org. Chem. (1974) 39:192, both of which are incorporated herein by reference.

In an alternative embodiment the positive reactive group is activated for reaction with reagents in solution. For example, a 5-bromo-7-nitro indoline group, when bound to a carbonyl, undergoes reaction upon exposure to light at 420 nm.

In a second alternative embodiment, the reactive group on the linker molecule is selected from a wide variety of negative light-reactive groups including a cinammate group.

Alternatively, the reactive group is activated or deactivated by electron beam lithography, x-ray lithography, or any other radiation. Suitable reactive groups for electron beam lithography include sulfonyl. Other methods may be used including, for example, exposure to a current source. Other reactive groups and methods of activation may be used in light of this disclosure.

As shown in Fig. 1, the linking molecules are preferably exposed to, for example, light through a suitable mask 8 using photolithographic techniques of the type known in the semiconductor industry and described in, for example, Sze, VLSI Technology, McGraw-Hill (1983), and Mead et al., Introduction to VLSI Systems, Addison-Wesley (1980), which are incorporated herein by reference for all purposes. The light may be directed at either the surface containing the protective groups or at the back of the substrate, so long as the substrate is transparent to the wavelength of light needed for removal of the protective groups. In the embodiment shown in Fig. 1, light is directed at the surface of the substrate containing the protective groups. Fig. 1 illustrates the use of such masking techniques as they are applied to a positive reactive group so as to activate linking molecules and expose functional groups in areas 10a and 10b.

The mask 8 is in one embodiment a transparent support material selectively coated with a layer of opaque material. Portions of the opaque material are removed, leaving opaque material in the precise pattern desired on the substrate surface. The mask is brought into close proximity with, imaged on, or brought directly into contact with the substrate surface as shown in Fig. 1. "Openings" in the mask correspond to locations on the substrate where it is desired to remove photoremovable protective groups from the substrate. Alignment may be performed using conventional alignment

techniques in which alignment marks (not shown) are used to accurately overlay successive masks with previous patterning steps, or more sophisticated techniques may be used. For example, interferometric techniques such as the one described in Flanders et al., "A New Interferometric Alignment Technique," App. Phys. Lett. (1977) 31:426-428, which is incorporated herein by reference, may be used.

To enhance contrast of light applied to the substrate, it is desirable to provide contrast enhancement materials between the mask and the substrate according to some embodiments. This contrast enhancement layer may comprise a molecule which is decomposed by light such as quinone diazid or a material which is transiently bleached at the wavelength of interest. Transient bleaching of materials will allow greater penetration where light is applied, thereby enhancing contrast. Alternatively, contrast enhancement may be provided by way of a cladded fiber optic bundle.

The light may be from a conventional incandescent source, a laser, a laser diode, or the like. If non-collimated sources of light are used it may be desirable to provide a thick- or multi-layered mask to prevent spreading of the light onto the substrate. It may, further, be desirable in some embodiments to utilize groups which are sensitive to different wavelengths to control synthesis. For example, by using groups which are sensitive to different wavelengths, it is possible to select branch positions in the synthesis of a polymer or eliminate certain masking steps. Several reactive groups along with their corresponding wavelengths for deprotection are provided in Table 1.

Table 1

Group	Approximate Deprotection Wavelength
Nitroveratryloxy carbonyl (NVOC)	UV (300-400 nm)
Nitrobenzyloxy carbonyl (NBOC)	UV (300-350 nm)
Dimethyl dimethoxybenzyloxy carbonyl	UV (280-300 nm)
5-Bromo-7-nitroindolinyl	UV (420 nm)
o-Hydroxy-α-methyl cinnamoyl	UV (300-350 nm)
2-Oxymethylene anthraquinone	UV (350 nm)

While the invention is illustrated primarily herein by way of the use of a mask to illuminate selected regions the substrate, other techniques may also be used. For example, the substrate may be translated under a modulated laser or diode light source. Such techniques are discussed in, for example, U.S. Patent No. 4,719,615 (Feyrer et al.), which is incorporated herein by reference. In alternative embodiments a laser galvanometric scanner is utilized. In other embodiments, the synthesis may take place on or in contact with a conventional liquid crystal (referred to herein as a "light valve") or fiber optic light sources. By appropriately modulating liquid crystals, light may be selectively controlled so as to permit light to contact selected regions of the substrate. Alternatively, synthesis may take place on the end of a series of optical fibers to which light is selectively applied. Other means of controlling the location of light exposure will be apparent to those of skill in the art.

The substrate may be irradiated either in contact or not in contact with a solution (not shown) and is, preferably, irradiated in contact with a solution. The solution contains reagents to prevent the by-products formed by irradiation from interfering with synthesis of the polymer according to some embodiments. Such by-products might include, for example, carbon

dioxide, nitrosocarbonyl compounds, styrene derivatives, indole derivatives, and products of their photochemical reactions. Alternatively, the solution may contain reagents used to match the index of refraction of the substrate. Reagents added to the solution may further include, for example, acidic or basic buffers, thiols, substituted hydrazines and hydroxylamines, reducing agents (e.g., NADH) or reagents known to react with a given functional group (e.g., aryl nitroso + glyoxylic acid → aryl formhydroxamate + CO_2).

Either concurrently with or after the irradiation step, the linker molecules are washed or otherwise contacted with a first monomer, illustrated by "A" in regions 12a and 12b in Fig. 2. The first monomer reacts with the activated functional groups of the linkage molecules which have been exposed to light. The first monomer, which is preferably an amino acid, is also provided with a photoprotective group. The photoprotective group on the monomer may be the same as or different than the protective group used in the linkage molecules, and may be selected from any of the above-described protective groups. In one embodiment, the protective groups for the A monomer is selected from the group NBOC and NVOC.

As shown in Fig. 3, the process of irradiating is thereafter repeated, with a mask repositioned so as to remove linkage protective groups and expose functional groups in regions 14a and 14b which are illustrated as being regions which were protected in the previous masking step. As an alternative to repositioning of the first mask, in many embodiments a second mask will be utilized. In other alternative embodiments, some steps may provide for illuminating a common region in successive steps. As shown in Fig. 3, it may be desirable to provide separation between irradiated regions. For example, separation of about 1-5 μm may be appropriate to account for alignment tolerances.

As shown in Fig. 4, the substrate is then exposed to a second protected monomer "B," producing B regions 16a and 16b. Thereafter, the substrate is again masked so as to remove the protective groups and expose reactive groups on A region 12a and B region 16b. The substrate is again exposed to monomer B, resulting in the formation of the structure shown in Fig. 6. The dimers B-A and B-B have been produced on the substrate.

A subsequent series of masking and contacting steps similar to those described above with A (not shown) provides the structure shown in Fig. 7. The process provides all possible dimers of B and A, i.e., B-A, A-B, A-A, and B-B.

The substrate, the area of synthesis, and the area for synthesis of each individual polymer could be of any size or shape. For example, squares, ellipsoids, rectangles, triangles, circles, or portions thereof, along with irregular geometric shapes, may be utilized. Duplicate synthesis areas may also be applied to a single substrate for purposes of redundancy.

In one embodiment the regions 12 and 16 on the substrate will have a surface area of between about 1 cm^2 and 10^{-10} cm^2. In some embodiments the regions 12 and 16 have areas of less than about 10^{-1} cm^2, 10^{-2} cm^2, 10^{-3} cm^2, 10^{-4} cm^2, 10^{-5} cm^2, 10^{-6} cm^2, 10^{-7} cm^2, 10^{-8} cm^2, or 10^{-10} cm^2. In a preferred embodiment, the regions 12 and 16 are between about 10x10 μm and 500x500 μm.

In some embodiments a single substrate supports more than about 10 different monomer sequences and perferably more than about 100 different monomer sequences, although in some embodiments more than about 10^3, 10^4, 10^5, 10^6, 10^7, or 10^8 different sequences are provided on a substrate. Of course, within a region of the substrate in which a monomer sequence is synthesized, it is preferred that the monomer sequence be substantially pure. In some embodiments, regions of the substrate contain polymer sequences which are at

least about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% pure.

According to some embodiments, several sequences are intentionally provided within a single region so as to provide an initial screening for biological activity, after which materials within regions exhibiting significant binding are further evaluated.

IV. Details of One Embodiment of a Reactor System

Fig. 8A schematically illustrates a preferred embodiment of a reactor system 100 for synthesizing polymers on the prepared substrate in accordance with one aspect of the invention. The reactor system includes a body 102 with a cavity 104 on a surface thereof. In preferred embodiments the cavity 104 is between about 50 and 1000 μm deep with a depth of about 500 μm preferred.

The bottom of the cavity is preferably provided with an array of ridges 106 which extend both into the plane of the Figure and parallel to the plane of the Figure. The ridges are preferably about 50 to 200 μm deep and spaced at about 2 to 3mm. The purpose of the ridges is to generate turbulent flow for better mixing. The bottom surface of the cavity is preferably light absorbing so as to prevent reflection of impinging light.

A substrate 112 is mounted above the cavity 104. The substrate is provided along its bottom surface 114 with a photoremovable protective group such as NVOC with or without an intervening linker molecule. The substrate is preferably transparent to a wide spectrum of light, but in some embodiments is transparent only at a wavelength at which the protective group may be removed (such as UV in the case of NVOC). The substrate in some embodiments is a conventional microscope glass slide or cover slip. The substrate is preferably as thin as possible, while still providing adequate physical support. Preferably, the substrate is less than about

1 mm thick, more preferably less than 0.5 mm thick, more preferably less than 0.1 mm thick, and most preferably less than 0.05 mm thick. In alternative preferred embodiments, the substrate is quartz or silicon.

The substrate and the body serve to seal the cavity except for an inlet port 108 and an outlet port 110. The body and the substrate may be mated for sealing in some embodiments with one or more gaskets. According to a preferred embodiment, the body is provided with two concentric gaskets and the intervening space is held at vacuum to ensure mating of the substrate to the gaskets.

Fluid is pumped through the inlet port into the cavity by way of a pump 116 which may be, for example, a model no. B-120-S made by Eldex Laboratories. Selected fluids are circulated into the cavity by the pump, through the cavity, and out the outlet for recirculation or disposal. The reactor may be subjected to ultrasonic radiation and/or heated to aid in agitation in some embodiments.

Above the substrate 112, a lens 120 is provided which may be, for example, a 2" 100mm focal length fused silica lens. For the sake of a compact system, a reflective mirror 122 may be provided for directing light from a light source 124 onto the substrate. Light source 124 may be, for example, a Xe(Hg) light source manufactured by Oriel and having model no. 66024. A second lens 126 may be provided for the purpose of projecting a mask image onto the substrate in combination with lens 112. This form of lithography is referred to herein as projection printing. As will be apparent from this disclosure, proximity printing and the like may also be used according to some embodiments.

Light from the light source is permitted to reach only selected locations on the substrate as a result of mask 128. Mask 128 may be, for example, a glass slide having etched chrome thereon. The mask 128 in one embodiment is provided with a grid of transparent

locations and opaque locations. Such masks may be manufactured by, for example, Photo Sciences, Inc. Light passes freely through the transparent regions of the mask, but is reflected from or absorbed by other regions. Therefore, only selected regions of the substrate are exposed to light.

As discussed above, light valves (LCD's) may be used as an alternative to conventional masks to selectively expose regions of the substrate. Alternatively, fiber optic faceplates such as those available from Schott Glass, Inc, may be used for the purpose of contrast enhancement of the mask or as the sole means of restricting the region to which light is applied. Such faceplates would be placed directly above or on the substrate in the reactor shown in Fig. 8A. In still further embodiments, flys-eye lenses, tapered fiber optic faceplates, or the like, may be used for contrast enhancement.

In order to provide for illumination of regions smaller than a wavelength of light, more elaborate techniques may be utilized. For example, according to one preferred embodiment, light is directed at the substrate by way of molecular microcrystals on the tip of, for example, micropipettes. Such devices are disclosed in Lieberman et al., "A Light Source Smaller Than the Optical Wavelength," Science (1990) 247:59-61, which is incorporated herein by reference for all purposes.

In operation, the substrate is placed on the cavity and sealed thereto. All operations in the process of preparing the substrate are carried out in a room lit primarily or entirely by light of a wavelength outside of the light range at which the protective group is removed. For example, in the case of NVOC, the room should be lit with a conventional dark room light which provides little or no UV light. All operations are preferably conducted at about room temperature.

A first, deprotection fluid (without a monomer) is circulated through the cavity. The solution preferably is of 5 mM sulfuric acid in dioxane solution which serves to keep exposed amino groups protonated and decreases their reactivity with photolysis by-products. Absorptive materials such as N,N-diethylamino 2,4-dinitrobenzene, for example, may be included in the deprotection fluid which serves to absorb light and prevent reflection and unwanted photolysis.

The slide is, thereafter, positioned in a light raypath from the mask such that first locations on the substrate are illuminated and, therefore, deprotected. In preferred embodiments the substrate is illuminated for between about 1 and 15 minutes with a preferred illumination time of about 10 minutes at 10-20 mW/cm^2 with 365 nm light. The slides are neutralized (i.e., brought to a pH of about 7) after photolysis with, for example, a solution of di-isopropylethylamine (DIEA) in methylene chloride for about 5 minutes.

The first monomer is then placed at the first locations on the substrate. After irradiation, the slide is removed, treated in bulk, and then reinstalled in the flow cell. Alternatively, a fluid containing the first monomer, preferably also protected by a protective group, is circulated through the cavity by way of pump 116. If, for example, it is desired to attach the amino acid Y to the substrate at the first locations, the amino acid Y (bearing a protective group on its α-nitrogen), along with reagents used to render the monomer reactive, and/or a carrier, is circulated from a storage container 118, through the pump, through the cavity, and back to the inlet of the pump.

The monomer carrier solution is, in a preferred embodiment, formed by mixing of a first solution (referred to herein as solution "A") and a second solution (referred to herein as solution "B"). Table 2

provides an illustration of a mixture which may be used for solution A.

Table 2
Representative Monomer Carrier Solution "A"

100 mg NVOC amino protected amino acid
37 mg HOBT (1-Hydroxybenzotriazole)
250 µl DMF (Dimethylformamide)
86 µl DIEA (Diisopropylethylamine)

The composition of solution B is illustrated in Table 3. Solutions A and B are mixed and allowed to react at room temperature for about 8 minutes, then diluted with 2 ml of DMF, and 500 µl are applied to the surface of the slide or the solution is circulated through the reactor system and allowed to react for about 2 hours at room temperature. The slide is then washed with DMF, methylene chloride and ethanol.

Table 3
Representative Monomer Carrier Solution "B"

250 µl DMF
111 mg BOP (Benzotriazolyl-n-oxy-tris(dimethylamino) phosphoniumhexafluorophosphate)

As the solution containing the monomer to be attached is circulated through the cavity, the amino acid or other monomer will react at its carboxy terminus with amino groups on the regions of the substrate which have been deprotected. Of course, while the invention is illustrated by way of circulation of the monomer through the cavity, the invention could be practiced by way of

removing the slide from the reactor and submersing it in an appropriate monomer solution.

After addition of the first monomer, the solution containing the first amino acid is then purged from the system. After circulation of a sufficient amount of the DMF/methylene chloride such that removal of the amino acid can be assured (e.g., about 50x times the volume of the cavity and carrier lines), the mask or substrate is repositioned, or a new mask is utilized such that second regions on the substrate will be exposed to light and the light 124 is engaged for a second exposure. This will deprotect second regions on the substrate and the process is repeated until the desired polymer sequences have been synthesized.

The entire derivatized substrate is then exposed to a receptor of interest, preferably labeled with, for example, a fluorescent marker, by circulation of a solution or suspension of the receptor through the cavity or by contacting the surface of the slide in bulk. The receptor will preferentially bind to certain regions of the substrate which contain complementary sequences.

Antibodies are typically suspended in what is commonly referred to as "supercocktail," which may be, for example, a solution of about 1% BSA (bovine serum albumin), 0.5% Tween in PBS (phosphate buffered saline) buffer. The antibodies are diluted into the supercocktail buffer to a final concentration of, for example, about 0.1 to 4 μg/ml.

Fig. 8B illustrates an alternative preferred embodiment of the reactor shown in Fig. 8A. According to this embodiment, the mask 128 is placed directly in contact with the substrate. Preferably, the etched portion of the mask is placed face down so as to reduce the effects of light dispersion. According to this embodiment, the imaging lenses 120 and 126 are not necessary because the mask is brought into close proximity with the substrate.

For purposes of increasing the signal-to-noise ratio of the technique, some embodiments of the invention provide for exposure of the substrate to a first labeled or unlabeled receptor followed by exposure of a labeled, second receptor (e.g., an antibody) which binds at multiple sites on the first receptor. If, for example, the first receptor is an antibody derived from a first species of an animal, the second receptor is an antibody derived from a second species directed to epitopes associated with the first species. In the case of a mouse antibody, for example, fluorescently labeled goat antibody or antiserum which is antimouse may be used to bind at multiple sites on the mouse antibody, providing several times the fluorescence compared to the attachment of a single mouse antibody at each binding site. This process may be repeated again with additional antibodies (e.g., goat-mouse-goat, etc.) for further signal amplification.

In preferred embodiments an ordered sequence of masks is utilized. In some embodiments it is possible to use as few as a single mask to synthesize all of the possible polymers of a given monomer set.

If, for example, it is desired to synthesize all 16 dinucleotides from four bases, a 1 cm square synthesis region is divided conceptually into 16 boxes, each 0.25 cm wide. Denote the four monomer units by A, B, C, and D. The first reactions are carried out in four vertical columns, each 0.25 cm wide. The first mask exposes the left-most column of boxes, where A is coupled. The second mask exposes the next column, where B is coupled; followed by a third mask, for the C column; and a final mask that exposes the right-most column, for D. The first, second, third, and fourth masks may be a single mask translated to different locations.

The process is repeated in the horizontal direction for the second unit of the dimer. This time, the masks allow exposure of horizontal rows, again 0.25 cm wide. A, B, C, and D are sequentially coupled using masks that expose horizontal fourths of the reaction area. The resulting substrate contains all 16 dinucleotides of four bases.

The eight masks used to synthesize the dinucleotide are related to one another by translation or rotation. In fact, one mask can be used in all eight steps if it is suitably rotated and translated. For example, in the example above, a mask with a single transparent region could be sequentially used to expose each of the vertical columns, translated 90°, and then sequentially used to allow exposure of the horizontal rows.

Tables 4 and 5 provide a simple computer program in Quick Basic for planning a masking program and a sample output, respectively, for the synthesis of a polymer chain of three monomers ("residues") having three different monomers in the first level, four different monomers in the second level, and five different monomers in the third level in a striped pattern. The output of the program is the number of cells, the number of "stripes" (light regions) on each mask, and the amount of translation required for each exposure of the mask.

Table 4

Mask Strategy Program

```
DEFINT A-Z
DIM b(20), w(20), l(500)
F$ = "LPT1:"
OPEN f$ FOR OUTPUT AS #1

jmax = 3        'Number of residues
b(1) = 3: b(2) = 4: b(3) = 5     'Number of building blocks for res 1,2,3
g = 1: lmax(1) = 1

FOR j = 1 TO jmax: g= g * b(j): NEXT j

w(0) = 0: w(1) = g / b(1)

PRINT #1, "MASK2.BAS ", DATE$, TIME$: PRINT #1,
PRINT #1, USING "Number of residues=##"; jmax
FOR j = 1 TO jmax
PRINT #1, USING "     Residue ##      ## building blocks"; j; b(j)
NEXT j
PRINT #1, "
PRINT #1, USING "Number of cells=####"; g: PRINT #1,

FOR j = 2 TO jmax
lmax(j) = lmax(j - 1) * b(j - 1)
w(j) = w(j - 1) / b(j)
NEXT j

FOR j = 1 TO jmax
PRINT #1, USING "Mask for residue ##"; j: PRINT #1,
PRINT #1, USING "   Number of stripes=###"; lmax(j)
PRINT #1, USING "   Width of each stripe=###"; w(j)
FOR l = 1 TO lmax(j)
a = 1 + (l - 1) * w(j - 1)
ae = a + w(j) - 1
PRINT #1, USING "   Stripe ## begins at location ### and ends at ###"; l; a; ae
NEXT l
PRINT #1,
PRINT #1, USING "   For each of ## building blocks, translate mask by ##
cell(s)"; b(j); w(j),
PRINT #1, : PRINT #1, : PRINT #1,
NEXT j
```